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Clinical epidemiology and diagnosis of bacterial vaginosis among pregnant women attending clinics in Irrua Specialist Teaching Hospital, Edo State, Nigeria

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ABSTRACT

Background: Bacterial vaginosis (BV) may be common in women of reproductive age group but little is known about the pattern of vaginal flora associated with BV in Nigeria sub-rural population.

Objectives: This study was designed to determine the prevalence, etiology, and standard diagnosis of bacterial vaginosis in Irrua Specialist Teaching Hospital Edo State, Nigeria. **Material and Methods**: This prospective study involved 344 consenting consecutive antenatal patients at the gestational age of 13-20 weeks attending Irrua Specialist Teaching Hospital Irrua, Edo State, Nigeria. Amsel diagnostic criteria were compared with Nugent's scores, the culture of Gardnerella vaginalis, and a combination of Nugent and culture of *Gardnerella vaginalis.*

Results: The prevalence of 30.23% was found using Amsel criteria, 22.09% using Nugent's method, and 23.26% from the culture of *Gardnerella vaginalis*. No statistical relationship was found between socio-demographic characteristics, sexual, social, and vaginal hygienic practices, and bacterial vaginosis. However, there was a statistical relationship between the report of fishy odor during and after sexual intercourse, *Gardnerella* morphotypes, Bacteriodes morphotypes, and

BV ($p<0.02$, $p<0.05$, $p<0.01$). There was an inverse relationship between lactobacilli morphotypes and BV. This study confirmed a strong relationship between Amsel criteria and Nugents method in the diagnosis of BV in pregnancy ($p=000$).

Conclusion: The prevalence rate of BV was high and the study highlights the polymicrobial nature and endemicity bacterial vaginosis among the studied population. Epidemiological risks popularly associated with BV did not seem to be important in the study.

Introduction

Bacterial vaginosis (BV) is the commonest cause of abnormal vaginal discharge in women of reproductive age, yet the etiology remains unclear (1, 2). It is a syndrome because no single bacterial agent can be regarded solely responsible for the syndrome and because of the absence of a true inflammatory response in most cases (1-5). The incidence varies in different parts of the world, e.g. 25% in a group of healthy Canadian women (6), 29.9% in Indonesia (7), 15% in rural Brazil (8), and 14.2% in healthy Nigerian women (9). An estimated 25-30% of women have BV at any given time mostly without signs and this rises to 85% in the prostitute population (10). The reported prevalence of BV among pregnant women ranges from 10-30% and in more than 30% of women that undergo termination of pregnancy in the United Kingdom (11).

Bacterial vaginosis is a polymicrobial superficial vaginal infection involving a reduction in the amount of hydrogen-peroxide producing lactobacillus and an overgrowth of anaerobic and gram-negative or gram – variable bacteria (12, 13). The reduced number of lactobacillus promotes the overgrowth of anaerobic bacteria including *Gardnerella vaginalis, Mycoplasma horminis, Bacteroides species, Mobiluncus species, privately species, Peptostreptococcus* Species (12-13). Mobiluncus species is a sensitive marker for the diagnosis of bacterial vaginosis (14). Women with bacterial vaginosis may experience an odorous discharge and/or abnormal vaginal bleeding, with one-half of cases being asymptomatic (3-5). On the other hand, Gardnerella vaginalis has been reported in up to a 50percent of women without symptoms or signs of BV making the bacteria not diagnostic of BV; (3, 15). The decrease in lactobacillus may be the most important predictor in subsequent BV development (16). Clue cells are formed when *Gardnerella Vaginalis*, present in high numbers, adhere in the presence of an elevated pH to exfoliated epithelial cells (18). In pregnancy, bacterial vaginosis is one of the leading causes of preventable preterm birth. A growing body of literature has begun to suggest an increased risk of spontaneous abortion among pregnant women with bacterial vaginosis (19-21). These infections are thought to contribute to preterm birth through complex interactions between micro-organisms and maternal and fetal natural defense mechanisms (22, 23). Sialidases are enzymes that play a role in bacterial nutrition, cellular interactions, and immune response evasion (24, 25). Sialidases are secreted from anaerobic gram-negative bacterial rods such as *Bacteroides*, *Gardnerella*, and *Prevotella* species (24-29). Pregnant women do not commonly develop bacterial vaginosis after 16weeks of gestation (30, 32). Classically, the diagnosis of BV is based on finding, three of the following four clinical criteria (Amsel's criteria) (31). Homogenous thin vagina fluid that adheres to the vaginal walls, vaginal pH greater than 4.5, whiff test; (release of amine or fishy odour with alkalinization (10% KOH), presence of Clue cell: (presence of vaginal epithelial cells with borders obscured with adherent small

bacteria) (33). Amsel criteria have the following limitations: Assessment of vaginal pH lacks specificity because an increase in vaginal pH may be a consequence of many other lower genital tract conditions. The conduct of the Whiff test is subjective for each clinician and lacks sensitivity and identification of clue cells may vary according to the microscopist and the quality of the sample (33).

Of the diagnostic methods currently available for assessment, the Amsel criteria is the Gold standard for the diagnosis of bacterial vaginosis and it reflects both the change in vaginal ecology and the strong microbial association (31). The method was modified by Nugent et al (36) to include the intermediate category that demonstrated the presence of mixed microbial flora with significant numbers of the lactobacillus morphotypes. The Nugent criteria is the test most often used in epidemiology while BV blue is a chromogenic point of care test based on detection of ion of increased vaginal fluid sialidase activity (>7.8IU) (39 41, 42). In non-pregnant women, the presence of bacterial vaginosis is associated with an increased risk of upper genital tract and sexually transmitted infections (2-4), and with the acquisition of HIV (5-9). In pregnancy, BV increases the risk of post-abortal sepsis, early miscarriage, recurrent abortion, late miscarriages preterm prelabour rupture of membrane (PROM), spontaneous preterm labour (SPTL), and preterm births, histological chorioamnionitis, and postpartum endometritis.

This study seeks to evaluate the prevalence of bacterial vaginosis in pregnancy in a semi-rural community in Nigeria using three of the known diagnostic methods (Amsel criteria, gram stain, and culture), assess the epidemiological profile and clinical correlates of BV, evaluate the vaginal bacterial microflora pattern of BV patients and validate Gram stain and culture diagnostic methods against the gold standard (Amsel Criteria).

Materials and methods

This was a descriptive cross-sectional study which was carried out at the Antenatal clinic, Irrua Specialist Teaching Hospital, Irrua, Edo State, a tertiary care hospital, and a referral center of Edo, Delta, Kogi, and Ondo States in Nigeria. The department has 52 gynecological and 58 Obstetrics beds and undertakes more than 1500 deliveries annually. The three hundred and twenty-three (323) patients sampled was guided by the upper limit required to give 95% level of confidence at an expected prevalence of 30%, using the precise prevalence formula: Sample size $N = Pq/(E/1.96)^2$ (78), where (1.96) is a constant, P is a maximum known prevalence of the disease (30%), q is 1-P (proportion of persons free from the disease) and E is the error margin allowable (5%). With the above formula, the minimum epidemiologically significant sample size to be collected was three hundred and twenty three (323). In other to account for sampling error and drop outs, the total sample collected was made up to three hundred and sixty three (363).

The study population was consecutive consenting pregnant women attending the antenatal clinic of Irrua Specialist Teaching Hospital, Irrua, Edo State. Consenting consecutive antenatal patients (who enrolled for antenatal care in the early second trimester from 13 up to 20weeks gestation regardless of symptoms and retroviral status) were recruited from August to December 2012. Pregnant women were excluded from the study for any of the following reasons; vaginal bleeding, use of lubricants or topical vaginal medications within the previous 72 hours antimicrobial therapy within 4 weeks, cervical cerclage, low lying placenta, steroids use, and pregnancy following assisted reproduction and diabetes mellitus in pregnancy. Gestational age was based on last menstrual period with corresponding height

measurement and ultrasound report. Participants were administered a structured interviewer's questionnaire which had 4 sections: Section A: Assessed the socio-demographic characteristics of the enrolled patients such as age, parity, marital status, level of education, husband's a profession/level of education, and ethnicity. Section B: Assessed the past reproductive performances eg; previous history of abortion, preterm delivery, puerperal sepsis, perinatal/neonatal infectious morbidity, STIs/HIV infection and intrauterine contraceptive device usage. Section C: Assessed the sexual, social and vaginal hygienic practices and current pregnancy. Section D: Contained the Performa designed for the study to record the results. The patients had genital examination done in dorsal position. Bivalve vaginal speculum was passed. No antiseptic lotions or creams were used for lubrication and where necessary, the vaginal speculum was moistened with sterile water. The vaginal wall was inspected and the presence of vaginal discharge and characteristics recorded. A pH paper (1-12 Merck & Co. Inc. Rahway, N.J) was mounted onto a 'mosquito' artery forceps which was gently introduced in the lateral wall/posterior fornix and was wetted with vaginal secretion. The pH was read and recorded. Two swab samples of the vaginal secretion were taken from the lateral wall or posterior fornix of the vagina using a plastic swab tipped with alginate wool in a peel pouch (medical wire and Equipment Co. Ltd; Corsham, Wilts, England). One of the swabs was used for Microscopy (wet preparation, gram stain) and the second swab was used for culture.

Following the removal of the vaginal speculum; 0.02ml (a drop) of 10% Potassium hydroxide (KOH) was added to the discharge on the speculum. The perception of a fishy amine odour was recorded as positive diagnosis for bacterial vaginosis (77). The presence of clue cells (>20%) was observed as the most closely associated criterion for the diagnosis of bacterial vaginosis. Clinical diagnosis of bacterial vaginosis was made using Amsel criteria (31).

During the clinical examination, direct smears were prepared gram stained using the kopeloff modification. Each microbial morphotype was measured and scored using Nugent's identification protocol (52-53) and a summary BV score computed (36) Large gram-positive bacilli were called the Lactobacillus morphotype. Small gram variable bacilli or coccobacilli were called the *Gardnerella* morphotype. Other organisms were categorized by morphology and interpreted accordingly. The swabs for culture were taken to the laboratory for processing using Amies transport medium. The transported vaginal swab was inoculated onto various selective and non-selective media. These solid media include blood agar, chocolate agar, McConkey agar, and saubouraud's agar. Columbia blood agar plates were incubated aerobically at 37^0C for 24 – 48 hours to isolate aerobic bacteria including lactobacilli. Columbia human blood agar plates were incubated at 36^0C and read after 48 – 72 hours for *Gardnerella vaginalis* isolation. The selective media for recovering gram-negative anaerobes from a specimen that may contain contaminating facultative flora were used i.e. Blood agar supplemented with neomycin (75ug/ml), Vancomycin (2.5ug/ml), and or nalidixic acid (10ug/ml). Identification was by carbohydrate fermentation and morphological analysis

Data were entered and stored in a Microsoft Excel Spreadsheet and analyzed using SPSS statistical package. Proportions were compared by Chi-square where appropriate and the statistical significance of p-value was $p<0.05$. Patients were excluded from the analysis where clinical information/specimens were not available.

Based on the results, the sensitivity; specificity, the false positive rate, positive prediction value, negative predictive value, and accuracy will be determined when the Amsel; composite criteria used as "gold standard" is compared with Gram stain, culture, and combination of Gram stain and culture. All sample collected for this study was treated with strict confidentiality Approval for the study was obtained from the ethical committee of the Irrua Specialist Teaching Hospital Irrua, Edo State, Nigeria

Results:

Three hundred and sixty-three consecutive pregnant women were enrolled in the study over five months between August and December 2012 regardless of symptoms after reviewing the pregnant women with features listed in the exclusion criteria but nineteen of them were disqualified due to incomplete data while 344 pregnant women's data were analyzed.

The mean age of the pregnant women was 27±4.55 years (range 17 - 38). The age range distribution shows that 6(1.74%) were in the age range <20years, 76 pregnant women (22.09%) were in the 20 – 24 years age range, 152 (44.19%) were in the 25 – 29 years age range, 76 (22.09%) were in 30-34 year age range while 34(9.88%) were in the age range >34 years. Parity ranged from 0 to 5. The mean was 1.058±1.19. The majority of the participants were nulliparous accounting for 136(39.53%), while primiparous women accounted for 156(45.34%).

Using the Amsel criteria, 104 (30.23%) of the women were diagnosed as having bacterial vaginosis in the study population. Age and parity did not significantly influence the occurrence of bacterial vaginosis in the study population. (X^2 00.0104, $\delta f = 4$, $p > 0.09$ for age $X^2 = 0.1515$, $\delta f = 4$, $p > 0.90$ for parity). Age less than 25years and low parity (0-2) also were not significantly associated with the diagnosis of bacterial vaginosis ($X^2 = 0.00491$ $\delta f = 1$ $p > 0.95$, for age <25, $X^2 = 0.13381$ $\delta f = 1$, $p > 0.7$ for parity <2).

Respondents' levels of education majority of them had secondary education as well as post-secondary education. One hundred and sixty-seven 167(48.54%) had post-secondary education, 104 (30.23%) completed secondary education, 49 (14.24%) of them had part secondary education. The level of education did not translate into improved financial status as the majority 112(32.56%) of them were unemployed. Level of education and occupation bore no statistical relationship with the diagnosis of bacterial vaginosis ($X^2 = 0.0012699$ $\delta f = 5$. $P > 0.95$ for education, $X^2 = 0.01516$ $\delta f = 6$, $P > 0.80$ for occupation.

The majority of the enrolled pregnant women 312(9.70) were married in a monogamous setting. Being single or cohabiting with a partner appeared to be associated with the diagnosis of bacterial vaginosis as 12 out of 16 of the single pregnant women had bacterial vaginosis. All the pregnant women cohabiting with a partner had bacterial vaginosis but there was no statistical relationship between marital status and diagnosis of bacterial vaginosis ($X^2 = 0.000145$, $\delta f = 5$, $P > 0.95$). Otherssocio–demographic characteristics were ethnicity and husband's occupation and level of education. The majority of them were Esan 232(67.44%), others were Yoruba 20(5.81%), Etsako 24(6.97%), Bini 16 (4.65%), Igbos 12(3.49%) Own 16(4.65%). The majority of the husband of the enrolled women had tertiary education and gainfully employed, unlike their wives.

Details about their past reproductive performance were collected through the interviewer questionnaire designed for the study, 196(56.98%) reported at least an aborted process of which 39(11.34%) were spontaneous abortion. 149(43.31%) had induced abortion, 12 (3.49%) had 2 episodes of previous spontaneous abortion while

8(2.32%) had three or more episodes of previous spontaneous abortion. There was no statistically significant relationship was found between the acquisition of bacterial vaginosis with the previous process (X^2 = 0.85993 δf = 1, P >0.5). table1

Only 25/344(7.27%) of the study population had one previous preterm delivery. Previous post-delivery post–aboral infection was also reported by 24 (6.98%) of the participants. No statistical relationship was found between previous preterm delivery, post-delivery about infections, previous sexually transmitted infection, previous intrauterine contraceptive device use, and diagnosis of bacterial vaginosis using Amsel criteria (table 1).

Table 2 showed sexual, social behavior, and vaginal hygienic practices in pregnancy in association with a clinical diagnosis of bacterial vaginosis. The mean age of sexual debut of the studies population was 18.36years 17.5± 4.95 years was the mean age of coitarche for women diagnosed to have bacterial vaginosis while 18.51± 2.519 years for negative bacterial vaginosis. Coital activity in pregnancy was high as 308(89.53%) of the enrolled pregnant women had sexual intercourse during pregnancy but no statistically significant relationship was found between sexual activity and clinical diagnosis of bacterial vaginosis (X^2 = 0.66857, δf = 1, P >0.5). Twice weekly sexual intercourse was reported 120(34.88%). There was a significant statistical relationship between the report of malodorous fishy smell during and after sexual intercourse with the diagnosis of bacterial vaginosis (X^2 = 5.22264, δf=1, p<0.02), forty-one (11.98%) had a new partner in present pregnancy, more than 40% had 2 or more partners before present pregnancy (table 3). Douching was reported by 111(32.27%) of the participants while 212 (61.62%) reported the use of medical soap or scented soap in washing the vagina (table 2).

Vaginal discharge was reported by 103 (29.94%) participants but on clinical examination, abnormal vaginal discharge was found in 160(46.59%) of the participants. The diagnosis of bacterial vaginosis was significantly dependent on finding of vaginal discharge on clinical pelvic examination and complaint of vaginal discharge (X^2 = 4.7948, δf =1, p<0.02, X^2 = 3.8301, δf=1, p<0.05) as shown in table 2. diagnosis of bacterial vaginosis clinically was not significantly dependent on douching, use of medicated/scented soap (X^2 = 0.69559, δf =1, p<0.03, X^2 = 0.001834, δf=1, p<0.90). Tables 3 and 4 showed vaginal microflora patterns in pregnant women with or without bacterial vaginosis as determined by gram stain smear of vaginal fluid and culture. The organisms seen on smears of the vaginal fluid showed that gram-positive cocci were seen in 71(20.06%) out of 104(30.23%) with BV and 5 (2.08%) out of 240 patients without bacterial vaginosis (X^2 = 4.6434, δf =1, p<0.05), Similarly, curved rods were seen in 20(19.93%) of 104(30.23%) with BV and in none of 240 patients without bacterial vaginosis (X^2 = 2.55591, δf =1, p<0.05). The *Gardnerella* morphotypes were seen more in cases of patients with BV (X^2 = 4.38470, δf =1, p<0.02). Small gram-negative bacilli resembling bactericides morphotypes were seen in 67(64.42%) out of 104 (30.23%) patients with BV and 14 (5.83%) out of 240 patients without BV (X^2 = 6.084315, δf =1, p<0.01). The lactobacilli morphotypes was absent or present only in low quality (1 to 2^+) in 81 (77.88%) out of 104(30.23%) with BV (X^2 = 4.86223, δf =1, p<0.05) and only 21(8.75%) of 240 patients without bacterial vaginosis

Table 5: shows the diagnostic composite criteria of Amsel. White thin homogenous discharge vaginosis and yellowish thick vaginal discharge were associated with a diagnosis of bacterial vaginosis. Among the diagnostic criteria, the presence of vaginal

discharge and clue cell greater than 20% were significantly associated with the diagnosis bacterial vaginosis (X^2 = 3.54709, δf =1, p<0.05, X^2 = 4.79485, δf =1, p<0.02 respectively).

Table 6: shows results of the Nugent's methods of diagnosis and its relationship with Amsel criteria. Among the enrolled women evaluated 104(30.23%) were diagnosed as positive and 240(69.77%) were negative based on clinical criteria. According to the gram stain using Nugent's method 76(22.09%) were deemed positive, 112(32.56%) had intermediate and normal finds were regarded as negative in this study. There was a significant relationship between the diagnosis using Amsel's criteria and abnormal Nugernt's score of (7-10. (X^2 = 9.3452, δf =1, p<0.001)

Table 7: Using the Amsel criteria as the "Gold Standard" for the diagnosis of bacterial vaginosis in pregnancy, there is evidence that Nugent's method has better sensitivity, specificity, positive predictive value, negative predictive value, and accuracy. 69.58%, 98.30%, 94.74%, 85.07%, 88.37% respectively) then the culture of *G. vaginalis* (26.92%) but the combination of culture and Nugent's method had a higher sensitivity (71.67%) but the accuracy of diagnosis is not improved. Gestational age for collection of specimen for the study was between 13 and 20 weeks with a mean of 16.24± 2.227 based on last menstrual period where necessary from ultrasound report.

TABLE 1: PAST REPRODUCTIVE PERFORMANCE

Clinical diagnosis				
	Total N = 344 (%)	Bacteria Vaginosis Positive (%)	Bacteria vaginosis negative (%)	P valu e
Abortion				
Yes	196(56.98)	60(17.44)	136(39.53)	
No	148(43.02)	44(12.79)	104(30/23)	>0.5
Type				
Spontaneous	39(11.34)	12(3.49)	27(7.85)	
Induced	149(43.31)	48(13.95)	101(29.36)	
Both	8(2.32)	0(0.00)	8(2.32)	NS
No of spontaneous				
1	19(5.52)	4(1.16)	15(4.36)	
2	12(3.49)	0.(0.00)	12(3.49)	>0.3
3 and above	8(2.32)	8(2.32)	0(0.00)	
Pre term delivery				
Yes	25(97.27)	8(2.32)	16(4.65)	
No	320(93.02)	96(27.9)	224(65.10)	NS
Previous post delivery/ abortal infection				

Yes	24(6.98)	8(2.32)	16(4.65)	NS
No	320(92.02)	96(27.9)	224(65.10)	
Previous neonatal morbidity / motality				
Yes	25(7.27)	4(1.16)	21(6.10)	NS
No	319(92.73(	99(28.80)	216(61.04)	
Previous sexually transmitted infection				
Yes	40(11.63)	16(4.65)	24(6.98)	Ns
No	319(92.73)	99(28.80)	216(61.04)	
Previous IUCD use	6	0(0.00)	6(1.74)	NS
Previous preterm delivery				
1	25(7.27)	8(2.33)	17(4.94)	
2 and more	0(0.00)	0(0.00)	0(0.00)	NS

Table 2
Sexual, social behavior and vaginal hygienic practices in pregnancy in relation to clinical diagnosis of bacterial vaginosis (using the Amstel criteria).

Clinical diagnosis				
	Total N = 344 (%)	Bacteria Vaginosis Positive (%)	Bacteria vaginosis negative (%)	P value
Vaginal Discharge as presenting complain				
Yes	103(29.94)	47(13.66).57	56(16.283)	
No	241(70.06)	57(16)	184(53.49)	>0.5
Vaginal discharge on clinical observation				
Yes	160(46.51)	100(29.07)	60(17.44)	
No	184(53.49)	1(0.29)	183(53.20)	<0.02
Age at coitache	18.36±SD	17.5±4.94	18.51±2.5192	
Coitus in pregnancy				
Yes	308(89.53)	92(27.44)	216(62.79)	
No	36(10.47)	12(0.87)	24(6.98)	>0.5
Frequency of coitus				
Daily	72(20.93)	24(6.98)	48(13.95)	
Twice weekly	120(34.88)	28(8.14)	92(27.44)	
Weekly	68(19.77)	24(6.98)	44(12.79)	
Occasionally	84(24.42)	28(8.14)	56(16.28)	NS
Malodorous fishy smell during and after intercourse				
Yes	36(10.47)	28(8.14)	8(2.33)	
No	308(89.53)	80 (25.25)	228 (2.33)	<0.02
No	308(89.53)	80(25.25)	228(66.28)	<0.02

Puritic vulva / vagina				
Yes	48	24(8.14)	24(6.98)	>0/95
No	296(86.04)	80(25.25)	216(62.79)	
New partner in current pregnancy				
Yes	4(11.98)	27(7.85)	14(4.07)	
No	303(88.08)	84(24.42)	219(63.66)	>0.3
No of partners before pregnancy				
1	204(59.30)	57(16.57)	147(42.73)	
2	80(23.26)	16(4.65)	64(18.61)	
≥3	60(17.44)	33(9.59)	27(7.85)	>0.99
Douching				
Yes	111(32.27)	32(9.30)	79(22.97)	
No	233(67.73)	72(20.93)	161(49.80)	>0.3
Use of medicated/scented soap for washing the vagina				
Yes	212(61.62)	77(20.38)	135(39.24)	>0.9
No	132(38.37)	27(7.85)	105(30.52)	
Retroviral status-positive	12	12	0	.000

Table 3: Vaginal microflora pattern in pregnant women with and without bacterial vaginosis as determined by Gram Stain smear of vaginal fluid.

Clinical diagnosis				
Morphotypes of organisms seen on gram stain	Total N = 344 (%)	Bacteria Vaginosis Positive (%)	Bacteria vaginosis negative (%)	P value
Gram positive cocci	76(22.09)	71(68.27)	5(2.08)	<0.05
Gram negative bacclli	81(23.54)	67(64.42)	14(5.83)	<0.01
Gram variable rods	24(6.97)	18(17.31)	6(2.50)	<0.02
Gram coccobacilli	56(16.27)	49(47.11)	7(2.91)	>0.05
Curved rods	20(5.81)	20(19.23)	0(0.00)	>0.05
Lactobacillus (gram positive rods) morphotypes $>2^+$	204(59.30)	3(2.88)	201(83.75)	>0.2
Lactobacillus (gram positive rods) morphotypes $0 – 2^+$	102(29.65)	81(77.88)	21(8.75)	<0.05
Gram negative cocci	5(1.45)	2(1.92)	3(1.25)	>0.7
Fusiform (bipolar rod)	8.(2.32)	7(6.73)	1(0.49)	>0.99
Yeast cells (bud, hyphae)	20(5.81)	8(7.69)	12(5.00)	>0.8

Table 4: vaginal microflora pattern in pregnant women with and without bacterial vaginosis as determined by cultivation

Clinical diagnosis				
Culture of organisms	Total N = 344 (%)	Bacteria Vaginosis Positive (%)	Bacteria vaginosis negative (%)	P value
Cultivation of organisms				
Lactobacilli spp	160(46.51)	12(11.53)	148(61.67)	<0.03

Gardneralla spp	80(23.26)	41(39.42)	39(11.33)	<0.01
Bacteriodes spp	64(18.64)	56(53.84)	8(2.32)	>0.01
Pepto-streptococci spp	57(16.57)	53(50.96)	4(1.66)	>0.2
Coliforms	8(2.32)	2(1.92)	6(2.50)	NS
Neisseria gonorrhea	4(1.16)	1(0.96)	3(1.25)	NS
Staphylococci spp	8(2.32)	2(1.92)	6(2.50)	NS
Prevotella spp	5(1.45)	5(4,80)	0(0.00)	>0.99
Unidentified gram negative rods	14(4.07)	14(13.46)	0.(0.00)	<0.01
Candida spp	30(8.72)	10(9.61)	20(8.33)	>0.7

Table 5: Nature of vaginal discharge and diagnosis of bacterial vaginosis using Amsel composite criteria

	Total N = 344 (%)	Bacteria Vaginosis Positive (%)	Bacteria vaginosis negative (%)	p value
Vaginal discharge on speculum examination				
Yes	160(45.51)	104(30.23)	56(16.28)	<0.02
No	184(53.49)	4(1.16)	180(52.32)	
Nature of vaginal discharge				
Homogenous discharge (white)	76(22.09)	56(16.28)	20(5.81)	
White thick/ curd like discharge	28(8.14)	8(2.32)	20(5,81)	
Yellowish thin discharge	24(6.96)	20(5.81)	4(1.16)	
Creamy discharge	20(5.81)	8(2.32)	12(2.49)	
Brownish discharge	4(1.16)	4(1.16)	0(0.00)	
pH≥ 4.5	148	68(19.77)	80(23.2)	>0.05
Whiff test	92(26.74)	84(25.58)	8(2.32)	>0.05
Wet preparation clue cell >20%	120(38.88)	88(25.58)	32(9.30)	<0.05
≥ 3 Amsel criteria	104(30.23)	104(30.23)	0.(0.00)	
< 3 Amsel criteria	240(69.77)	0.(0.00)	240(69.77)	

Table 6: The relationship between Gram stain Nugent's method and Amsel criteria

Gram stain	Nugent score	Total N = 344 (%)	Bacteria Vaginosis Positive (%) N= 104	Bacteria vaginosis negative (%) N= 240	P value
Normal	0 – 3	156(45.35)	4(3.85)	152(63.33)	
Intermediate (mixed flora)	4 – 6	112 (32.56)	36(34.62)	76(31.66)	
Abnormal	7 – 10	76(22.09)	72 (69.23)	4(1.67)	<0.001

Table 7: Comparison of sensitivity, specificity, predictive values, positive and negative rates of gram stain, culture and both with clinical diagnosis using Amsel criteria.

Sensitivity (%)	Specificity (%)	PPV (%)	NPV(%)	FPR (%)	FNR (%)	Accuracy (%)

Nugent' method (gram stain)						
69.58	98.3	94.74	85.07	1.72	22.09	88.37
Culture of G. Vaginalis						
26.92	78.33	35.00	71.21	21.66	28.78	62.79
Combination of Nugent's methods and culture of G. vaginalis						
71.42	85.18	38.46	95.83	14.81	4.16	59.30

**Gram stains were scored using Nugent's methods; intermediate scores were considered negative. *An Amsel criteria positive result required positive results for three of the four criteria PPV = positive predictive value, NPV = negative predictive value, FPR = false positive value, FNR = false negative value

Discussion

A prevalence of 30.23%, 22.09% using Nugent's method, and 23.26% from the culture of *Gardnerella vaginalis* was found in the studied population. This compares to the range of 20-23% in Burkina Faso (54) and Malawi (55) and 20-30% in Kenya and South Africa (58-59). The prevalence rate in this study is higher than a three Nigeria study with 10%, 17%, and 1.2% respectively (60-62).

Ages less than 25 years did not influence low parity (0-2), while a low level of education did not determine the occurrence of bacterial vaginosis in pregnancy

Association between bacterial vaginosis and sexual risk factors has been reported in both heterosexual and homosexual women, however, the notion that bacterial vaginosis is sexually transmitted is still debated. In this study, pregnant women with new partners in current pregnancy (27/41), a higher number of lifetime partners before pregnancy especially greater than three, early coitarche, and previous STI were not associated with the diagnosis of bacterial vaginosis. This is at variance with the findings of other authors (64-66)

Douching has been linked to bacterial vaginosis as well as to Chlamydia cervicitis in some women but not in all studies (67). In this study, the use of medicated soap and douching was not significantly ($p<0.05$) associated with the frequency of bacterial vaginosis in pregnancy.

This study found a strong statistical significance between abnormal (Nugent's method) score with clinical diagnosis of bacterial vaginosis (X^2 = 9.2452, df =1, $p>0.001$). There is also a significant positive association between anaerobic morphotypes, gardneralla morphotypes (X^2 = 4.6434, df =1, $p>0.05$, X^2 = 4.2847, df =1, $p>0.02$, X^2 = 6.0832, df =1, $p>0.01$) and clinical diagnosis using Amsel criteria which is similar to what has been reported by various authors(35,36,38,52,68).

A lower concentration of facultative species of lactobacilli among pregnant women with BV in comparison to women with a normal flora was noted in this study. A low pH has been shown to have direct microbicidal and veridical effects (65). Lactobacillus species can also adhere to the vaginal epithelial cells blocking the attachment of any pathogenic BV-associated bacteria onto these cells. Lactobacilli are known for the maintenance of a healthy vaginal microenvironment (70-72).

Several logistic problems arose in the course of this study which may limit the interpretation of data on vaginal flora pattern. First mycoplasma hominis and ureaplasma urealyticum were not isolated because appropriate culture media were not available.

Mobiluncus spp were not isolated despite observing Mobiluncus species-like organism (curved rods) is some vaginal gram smears. These findings could be attributed to a low prevalence of Mobiluncus species in our studied population or to an inadequate isolation procedure that will warrant further investigation.

Other bacteria isolates in this study were Staphylococcus spp 8(2.32%), Coliforms 8(2/32%), Neisseria gonorrhea 4(1.16%), and different species of Candida characteristics by their buds, hyphae, and chromotubation 30(8.72%). The finding of Neisseria gonorrhea 37 is not surprising because increasing data also indicate that BV facilitates the acquisition of sexually transmitted diseases including Neisseria gonorrhea, HIV, HSV type 2, and Chlamydia trachomatis. Following microbiological analysis, Gardnerella vaginalis was isolated in 80(23.26%) of the enrolled pregnant women but it was only isolated in about 41(39.42%) of the patients diagnosed to have BV using Amsel criteria. This is not surprising because Gardnerella vaginalis is also regarded as part of normal vaginal flora and this has been demonstrated by several authors.69-72

The presence of Gardnerella vaginalis was not restricted to women with clinical signs of BV. Gardnerella vaginalis was isolated in both the normal and intermediate groups in this study. It has been postulated that a synergistic mechanism exists among the bacteria involved in BV (73). Current findings revealed a positive correlation between Gardnerella vaginalis and Prevotella involving ammonia utilization and also between prevolella and Peptostreptococcus species.

Using Nugent's method 112(32.56%) vaginal flora has intermediate-grade or scores of 4-6. This has been described as a mixed microbial flora acting as a transitional phase between normal and BV flora. Studies have shown that subsequent sampling of women in this intermediate grade revealed that some transition to normal flora and other acquire BV (74). The emergent of this intermediate phase will require additional study to determine the factors that influence the vaginal microflora that lead to the initial overgrowth of Gardneralla vaginalis and subsequent increase in anaerobic organisms. The presence of intermediate flora has been shown to increase the risk of adverse obstetric outcomes and acquisition of HIV (75, 76).

There was a statistical significant relationship between vaginal discharge as presenting complaint (on clinical observation (X^2 = 4.7949, df =1, p>0.02), vaginal discharge on clinical observation (X^2 = 5.6302, df =1, p>0.01) and diagnosis of bacteria vaginosis using Amsel Criteria. While 103(29.94%) of the patients reported to no vaginal discharge on questioning, 160(46.51%) had it on clinical observation (X^2 = 0.02815, df =1, p>0.8) this disagreed with the work done by Apea Kubi et al (63), in which there was a significant relationship between presenting symptoms and clinical observation of vaginal discharge. The nature of the discharge does not seem to correlate with the diagnosis of BV. In this study, white homogenous and yellowish thick vaginal discharge was more associated with BV. Gardner et al (34) described a thin grey homogenous discharge with a tendency to adhere to the vaginal well rather than pool in the posterior fornix was found to be associated with BV. However, Thomason et al (37) found homogenous discharge to be of little value in diagnosing BV, while Krohn et al (27) showed that in pregnant women, homogenous discharge was not independently related to bacterial vaginosis. The study further highlights the importance of microbiological examination

of vaginal discharge over and above clinical observation.

The presence of clues of cells detected in a wet preparation of vaginal fluid correlated well with a clinical diagnosis of BV. This is not surprising since the presence of clue cells greater than 20% was one of the four criteria used to define BV. The pH≥ 4.5 was reported in this study in up to 148(43.02%) of cases but only 68(19.77%) of cases were diagnosed to have BV clinically. This is because it's affected by a lot of factors like recent intercourse because of the release of alkaline semen, cervical mucus, blood, trichomoniasis. High pH 5 and 6 are said to promote adherence of G. vaginalis and anaerobic organism to vaginal epithelial cells. Whiff test (positive amine test) was observed in 92(26.74%) of the pregnant women and 84(24.42%) were diagnosed to have BV clinically. This is not surprising because organisms associated with BV produces amino and malic acids which irritates mucus membrane and fishy odor during intercourse or following the addition of 10% KOH of vagina fluid, in this study, report of malodorous fishy odor during and after intercourse was significantly associated with the clinical diagnosis of bacterial vaginosis ($X^2 = 5.2226$, df =1, $p>0.02$).

In this study, normal gram stain using Nugent's method correlated with clinical diagnosis in 72(69.23%) of cases diagnosed clinically. Up to 36 (34.64%) with intermediate flora diagnosed clinically to have BV using Amsel criteria. BV was diagnosed in 12(100%) clinically using Amsel criteria while using Nugent's method, 4(33.33%) have BV, and 8(66.67%) had intermediate flora.

Nugent's method compared with the "Gold standard" as used in this study had a sensitivity of 69.2% specificity of 95%, negative predictive value 85.07%, positive predictive value 94.74%, false-positive rate of 1.72%, false-negative rate 22.09%, and accuracy of 88.37%. This study found a strong association between Amsel criteria and Nugent's scores, and the isolation of G. vaginalis and anaerobic organisms, and an inverse relationship with the presence of lactobacilli. However, few cases diagnosed clinically using Amsel criteria were missed by Nugent's method. The finding of intermediate flora by Gram stain was similar to the corresponding population in Nigeria (89) and other countries in Africa (58, 59) and the pattern of vaginal microflora associated with BV were also similar. Amsel criteria and Nugent's method are recommended for use clinically in Nigeria especially as they correlated well with the result of polymerase chain reaction technology in the developed world (9). Direct Gram staining of smear should facilitate the diagnosis of BV for confirmation by culture, particularly in a third world setting with a few standard laboratory facilities (68).

In conclusion: the finding of the study is instructive in many respects. The incidence of bacterial vaginosis is high among the antenatal population. The overall contribution to adverse pregnancy outcomes as it relates to preterm delivery, low birth weight chorioamnionitis, and neonatal infectious morbidity can only be inferred from findings done by other workers. Amsel criteria remain the gold standard in making a diagnosis. The study highlights the polymicrobial nature of the condition rather than any specific organism. The epidemiological risk factors popularly associated with BV did not seem from this study to be important. This may be explained by the subset of the population used. There was also from this study a strong correlation between symptoms and microbiological diagnosis. This makes a compelling case for empirical treatment especially in the third world environment. This is because of the dearth of appropriate laboratory facility and trained personnel. Microbiological

confirmation however should always be aimed at and this study has confirmed the high degree of correlation between the Amsel criteria and Nugent's score. Future longitudinal studies are needed to evaluate adverse pregnancy outcome associated with the especially high incidence of BV in this environment. This strategy will in no small way improve pregnancy outcome in this environment.

CONFLICT OF INTEREST

There was no conflict of intrest

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